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Metabolic status, gonadotropin secretion, and ovarian function during acute nutrient restriction of beef heifers[1,2]

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ABSTRACT: The effect of acute nutritional restriction on metabolic status, gonadotropin secretion, and ovarian function of heifers was determined in 2 experiments. In Exp. 1, 14-mo-old heifers were fed a diet supplying 1.2 × maintenance energy requirements (1.2M). After 10 d, heifers were fed 1.2M or were restricted to 0.4 × maintenance requirements (0.4M; d 0). Heifers received $PGF_{2\alpha}$ (25 mg, intramuscularly) on d –10, 0, and 10 to synchronize ovulation. After 30 d, 1.2M and 0.4M heifers were realimented to 1.2 M for 100 d. Blood samples were collected every other day from d 0 to 14 then 3 times weekly thereafter. Heifers in Exp. 2 were managed as in Exp. 1 except that animals were fitted with an indwelling jugular catheter and blood samples were collected at 10-min intervals for 8 h on d 9, 10, and 11. Concentrations of progesterone in plasma were used to quantify ovarian luteal function. All 1.2M heifers ovulated, whereas only 30% of 0.4M heifers ovulated in Exp. 1. Concentrations of NEFA were greater and concentrations of thyroxine and IGF-I were less ($P < 0.05$) in plasma of 0.4M heifers compared with 1.2M heifers. The size of dominant follicles in Exp. 1 was reduced ($P < 0.05$) in 0.4M compared with 1.2M heifers. Concentrations of IGF-I were increased and anovulatory heifers resumed ovarian cycles an average of 35 d after realimentation. Concentrations of insulin were greater ($P < 0.05$) in plasma of 1.2M compared with 0.4M heifers in Exp. 2. The frequency of LH pulses was reduced ($P < 0.05$) in 0.4M heifers on d 9, and FSH in plasma on d 11 was not influenced by treatment. Reduced concentrations of IGF-I in plasma of nutrient-restricted heifers were associated with the reduced size of dominant follicles and indicated a local effect of growth factors on follicles. The decreased LH pulse frequency of 0.4M heifers before luteolysis indicates that restriction of nutrients decreased LH support of follicle growth. A preovulatory increase in estradiol in plasma and an ovulatory surge of LH were not detected in nutrient restricted heifers that did not ovulate. It is concluded that restricting beef heifers to 0.4 × maintenance energy requirements reduced the availability of metabolic fuel and decreased metabolic hormones, resulting in changes within the reproductive neuroendocrine-ovarian axis that compromised the ability of the dominant follicle to secrete sufficient concentrations of estrogen to stimulate an ovulatory surge of LH.

Key words: heifer, insulin-like growth factor-I, luteinizing hormone, nutrition, ovary, reproduction

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INTRODUCTION

The reproductive axis of young animals is regulated by nutrient availability and is sensitive to shifts in metabolism (Barb et al., 1997; Amstalden et al., 2000). Estrous cycles occur in heifers with moderate caloric restriction (loss of 11% BW in 35 d; Imakawa et al., 1986), but anovulation occurs only after prolonged duration of limited nutrient intake (20 wk, Rhodes et al., 1996; 32 wk, Bossis et al., 1999). Because acute nutritional restriction in cattle is equalized by the rumen, it is debatable as to whether the reproductive axis of cyclic heifers is sensitive to acute nutritional restriction. Mackey et al. (1999) reported that programmatic feeding resulting in nutritional restriction (40% of maintenance) for 14 d acutely suppressed follicular growth and induced ovulatory failure in 60% of heifers; estradiol in dominant follicles was reduced (Walsh et al., 2012a, 2012b). However, Lents et al. (2011) found that a similar acute nutritional restriction did not suppress ovulation in heifers.

Feed restriction of heifers reduces growth and size of dominant follicles (Rhodes et al., 1996; Bossis et al., 1999) and suppresses secretion of LH (Day et al., 1986; Bossis et al., 1999; Amstalden et al., 2000) because of reductions in GnRH release from the hypothalamus (Bishop and Wettemann, 1993). Altered hypothalamic-pituitary-ovarian function was accompanied by altered concentrations of glucose, insulin, NEFA, leptin, and IGF-I in plasma (Amstalden et al., 2000; Bossis et al., 2000; Lents et al., 2011). These metabolic factors may provide both short- and long-term regulation of LH secretion and follicular growth in cattle (Wettemann and Bossis, 2000; Diskin et al., 2003). The current studies were conducted to test the hypothesis that nutritional restriction of heifers to 40% of maintenance requirements for 14 d reduces pulsatile secretion of LH during development of the preovulatory follicle and inhibits normal cyclic ovarian function. The objective was to quantify metabolic and endocrine function associated with ovarian response to acute nutritional restriction and realimentation.

MATERIALS AND METHODS

Animals and Procedures

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Oklahoma State University and were conducted in accordance to the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (Federation of Animal Science Societies, 2010).

Table 1. Ingredient composition and composition in diet on DM basis

Item	Amount
Ingredient, % of DM	
Rolled corn	40.0
Alfalfa pellets	35.0
Cottonseed hulls	21.75
Molasses	3.0
Trace mineralized salt	0.25
Nutrient composition[1]	
DM, %	88.77
NE_m, Mcal/kg	1.62
NE_g, Mcal/kg	0.87
Fat, %	3.11
ADF, %	28.25
NDF, %	38.72
CP, %	10.71

[1] On a DM basis, dry matter intake: Exp. 1, 1.2M = 4.37 kg, 0.4M = 1.44 kg; Exp. 2, 1.2M = 5.07 kg, 0.4M = 1.69 kg, where 1.2M = 1.2 × maintenance energy requirements and 0.4M = 0.4 × maintenance requirements.

Experiment 1

Animals and Management. Angus × Hereford heifers (n = 19) at 14 mo of age (317 ± 17 kg; BCS = 5.7 ± 0.3, 1 = emaciated and 9 = obese; Wagner et al., 1988) and exhibiting normal estrous cycles were housed in individual pens in an environmentally controlled building and adapted to a diet (Table 1) supplying 1.2 × maintenance energy requirements (**1.2M**) for 10 d. The amount of feed for 0.4 × maintenance energy requirements (**0.4M**) and 1.2M was determined for each heifer with OSUNRC software (http://www.beefextension.com/files/OSUNRC2010%20Excel%202007%20final.xlsx). The NE_m was calculated as 0.077 Mcal/$BW^{0.75}$ because it is derived using data from heifers of British breeds penned in generally nonstressful environments (Garrett, 1980). On d 0, heifers were randomly assigned to continue receiving 1.2M (n = 9) or were fed a restricted amount of feed to supply 0.4M (n = 10) for 29 d. This experiment consisted of 2 replicates with 12 heifers in replicate 1 (n = 6 heifers for each treatment) and 7 heifers in replicate 2 (n = 3 heifers for 1.2M and n = 4 heifers for 0.4M). Replicate 1 was initiated in October, and replicate 2 started in the subsequent September. All heifers were exposed to similar feed and management before the experiment. On d 30, 0.4M heifers were adapted to 1.2M over a 10-d period (5 d at 0.6M and 5 d at 0.8M), and then all heifers were fed 1.2M for 90 d (100 d of realimentation).

Estrous Synchronization and Determination of Ovulation. All heifers received $PGF_{2\alpha}$ [25 mg, intramuscularly (**i.m.**); Lutalyse, Pfizer Animal Health, New York, NY] at the start of the 10-d adaptation period (d –10), on d 0, and on d 10 to induce regression of

corpora lutea (**CL**) and induce ovulation of dominant follicles. Because animals were maintained in individual pens, estrous behavior was not observed. Heifers with plasma progesterone concentrations <0.5 ng/mL from d 14 to 21 were defined as anovulatory (Wettemann et al., 1972). Transrectal ultrasound (7.5-MHz probe, Corometrics Medical Systems, Wallingford, CT) was used to measure the size of the largest follicle on the ovaries of heifers from d 7 to 14 of treatment. Follicle size was calculated as the mean of the longest and shortest diam. (Ciccioli et al., 2003; Lents et al., 2008; White et al., 2008).

Blood Sampling and Assays. Blood samples were collected before feeding at 0800 h by tail venipuncture every other day from d 0 to 14 and then 3 times per week thereafter. Blood samples were collected into 10-mL tubes containing EDTA [0.1 mL of a 15% solution (wt/vol)] and placed on ice until plasma was obtained by centrifugation (2,500 × *g* at 4°C for 15 min) within 2 h. Plasma was decanted and stored at –20°C until analyses.

Concentrations of progesterone in plasma were quantified by solid-phase RIA (Siemens Healthcare Diagnostics, Tarrytown, NY) validated for use in bovine plasma (Vizcarra et al., 1997). Intra- and interassay CV were 6% and 7%, respectively. Plasma concentrations of IGF-I were determined by RIA (Echternkamp et al., 1990) after acid-ethanol extraction (16 h, 4°C). Recombinant human IGF-I (R&D Systems, Minneapolis, MN) was used for standards. Intra- and interassay CV were 6% and 8%, respectively. Concentrations of insulin in plasma were determined by solid-phase RIA for human insulin (Siemens Healthcare Diagnostics) using bovine pancreatic insulin (Sigma Chemical Co., St. Louis, MO) for standards as previously validated (Bossis et al., 1999). Intra- and interassay CV were 9% and 11%, respectively. Concentrations of thyroxine were determined by solid-phase RIA (Siemens Healthcare Diagnostics) validated for use with bovine plasma (Ciccioli et al., 2003). The intra- and interassay CV were 5% and 9%, respectively. Concentrations of glucose in plasma were quantified by an enzymatic colorimetric procedure (Sigma Chemical Co.) in a single assay with an intra-assay CV of 8%. Concentrations of NEFA were determined in a single assay by an enzymatic colorimetric procedure (Wako Chemicals Inc., Dallas, TX) with modification (McCutcheon and Bauman, 1986); intra-assay CV was 5%. Concentrations of plasma leptin were quantified in a single RIA specific for ovine leptin and validated for use in bovine serum (Delavaud et al., 2000). The intra-assay CV was 8.5%.

Statistical Analyses. Chi-squared analysis (SAS Inst. Inc., Cary, NC) was used to evaluate the effect of treatment on the percentage of heifers that became anovulatory. A mixed-model ANOVA (PROC MIXED, SAS) for repeated measures was used to determine effects of treatment (1.2M vs. 0.4M) on daily concentrations of glucose, NEFA, and hormones. The model included the fixed effects of treatment and day, with cow within day (d 0 to 14 or d 30 to 100) as the repeated unit. The within-animal covariance structure for the repeated measure was modeled by a first-order autoregressive function with lag equal to 1. Degrees of freedom for the pooled error term were calculated using Kenward-Roger's approximation, and the pooled error term was used to test the effect of day. Plasma concentrations of glucose, insulin, NEFA, and thyroxine were measured during restricted feeding in the first replicate only, whereas concentrations of IGF-I and leptin in plasma were quantified during restriction and realimentation in both replicates 1 and 2. Concentrations of insulin were not assayed on d 12 of restriction; therefore, d 12 was not included in this model. Samples were not collected on d 4 of restriction during the second replicate. The random effects of replicate and assay were included in the model when appropriate. Student's *t* test was used to compare means when treatment or day effects were significant.

Experiment 2

Animals and Management. Angus × Hereford heifers (n = 23) at 15 mo of age (BW = 387 ± 7 kg; BCS = 5.5 ± 0.1) and exhibiting normal estrous cycles were housed in individual pens in September and adapted to the 1.2M diet (Table 1) for 10 d. On d 0, heifers were randomly assigned to diets of 0.4M (n = 15) or 1.2M (n = 8) for 21 d. Amount of feed was calculated as described for Exp. 1. After blood samples were collected on d 12, ten heifers (n = 6 for 0.4M, and n = 4 for 1.2M) were randomly selected for exsanguination to meet other experimental objectives not reported.

All heifers received $PGF_{2\alpha}$ (25 mg) at the start of the 10-d adaptation period (d –10) and on d 0 and d 10 to induce regression of CL and allow ovulation of dominant follicles. Because animals were maintained in individual pens, estrous behavior was not observed. Ovulation was based on progesterone in plasma as described for Exp. 1.

Blood Sampling and Assays. Blood samples were collected on d 0 to 21 as described for Exp. 1. On d 8, a polyvinyl cannula (1.68 mm i.d., 2.39 mm o.d.; Bolab Inc., Lake Havasu City, AZ) was inserted into a jugular vein of each heifer, and animals were confined to stalls. Serial blood samples (10 mL) were collected every 10 min for 8 h on d 9, 10, and 11. Blood samples were allowed to clot for 24 h at 4°C and centrifuged (2,000 × *g* at 4°C for 30 min), and serum was decanted and stored at –20°C until analyzed. Commencing at 1200 h on d 12, serum and plasma samples were collected from 0.4M (n = 9) and 1.2M (n = 4) heifers every 4 h for 48 h to quantify the proestrus increase in estradiol-17β and the ovulatory surge of LH.

Plasma concentrations of progesterone, insulin, and IGF-I were quantified as described for Exp. 1. Concentrations of estradiol-17β in plasma were determined in a single assay by RIA (Serono, Biodata SpA, Montecelio, Italy) with modifications (Vizcarra et al., 1997). Intra-assay CV was 12%. Serum concentrations of LH were quantified by RIA (Bishop and Wettemann, 1993) with NIH-bLH-B9 for standards. Intra- and interassay CV were 11% and 15%, respectively. Concentrations of FSH in serum were quantified by RIA (Vizcarra et al., 1997) with USDA-bFSH-I-2 for standards. Intra- and interassay CV were 10% and 21%, respectively. All samples of an individual animal were blocked by treatment within assay, and individual samples were randomized within each assay.

Statistical Analyses. Data from one 1.2M heifer that was exsanguinated on d 12 were not included in the statistical analyses because she became lame. Chi-squared analyses were used to evaluate the effect of treatment (0.4M vs. 1.2M) on the percentage of heifers that ovulated. A mixed-model ANOVA (PROC MIXED, SAS) for repeated measures was used to determine effects of treatment (1.2M vs. 0.4M) on daily concentrations of hormones. The model included the fixed effect of treatment with day as the repeated unit and assay as a random effect. The within-animal covariance structure for the repeated measure was modeled by a first-order autoregressive function with lag equal to 1. Degrees of freedom for the pooled error term were calculated using the Kenward-Roger approximation, and the pooled error term was used to test the effect of day. Fisher's LSD was used to compare means when F tests for treatment or day effects were significant ($P < 0.05$).

Frequency and amplitude of LH and FSH pulses were determined using the PC pulsar program (Merriam and Wachter, 1982) with these G values: for LH, G1 = 99, G2 = 3.23, G3 = 2.75, G4 = 2.25, and G5 = 99; for FSH, G1 = 99, G2 = 3.23, G3 = 3.00, G4 = 2.50, and G5 = 99. The G values were chosen to serve as criteria to determine whether variations in hormone concentrations in serial samples are pulses in hormone secretion or just random variation in concentrations. The effects of treatment on mean concentration, frequency of pulses, and amplitude of pulses of LH (on d 9, 10, and 11) and FSH (d 11) were determined by ANOVA using the mixed model described above.

RESULTS

Experiment 1

All heifers had similar BCS ($P = 0.80$) and BW ($P = 0.61$) before treatment. Body condition score was similar ($P = 0.80$) between 0.4M and 1.2M heifers after 14 d of treatment, but 0.4M heifers lost an average of 15 kg of BW by d 14, whereas 1.2M heifers maintained BW ($P < 0.001$; Table 2). The size of the dominant follicle was reduced in 0.4M compared with 1.2M heifers (Fig. 1; $P = 0.05$). All 1.2M heifers ovulated (9 of 9), as determined by plasma concentrations of progesterone from d 14 to 21, whereas only 30% of the 0.4M heifers (3 of 10) ovulated ($P < 0.001$; Table 2).

Table 2. Initial and final BW and BCS, change in BW, and number of heifers that were ovulatory after 21d of nutritional treatment

	Dietary treatment[1]		SEM
Item	1.2M	0.4M	
Experiment 1			
No.	9	10	—
Initial BW,[2] kg	320	314	7
Final BW,[2] kg	323[a]	299[b]	5
Change in BW, kg	3[a]	–15[b]	3
Initial BCS	5.7	5.7	0.1
Final BCS	5.4	5.3	0.2
Ovulating,[3] no.	9 of 9	3 of 10	—
Experiment 2			
No.	7	15	—
Initial BW,[4] kg	388	388	7
Final BW,[4] kg	392[a]	353[b]	8
Change in BW, kg	4[a]	–35[b]	4
Initial BCS	5.5	5.4	0.1
Final BCS	5.5	5.3	0.1
Ovulating,[5,6] no.	4 of 4	5 of 9	—

[a,b]Means within a row without a common superscript are different ($P < 0.01$).

[1]Animals received 1.2 times (1.2M) or 0.4 times (0.4M) their maintenance energy requirement for 29 d in Exp. 1 and 21 d in Exp. 2.

[2]Initial and final BW and BCS determined on d 0 and 14 from diet allocation, respectively.

[3]Chi-squared distribution ($P < 0.001$).

[4]Initial and final BW and BCS were determined on d 0 and 12 from diet allocation, respectively.

[5]On d 12 from diet allocation, 4 of the 1.2M heifers and 6 of the 0.4M heifers were sacrificed, and their ovulatory status is unknown.

[6]Chi-squared distribution ($P = 0.10$).

During restricted feeding, there was a treatment × day effect for NEFA ($P < 0.01$) and IGF-I ($P < 0.001$) but not for glucose ($P = 0.40$), insulin ($P = 0.18$), thyroxine ($P = 0.80$), or leptin ($P > 0.1$). Concentrations of NEFA in plasma were greater ($P < 0.01$) for 0.4M heifers than for 1.2M heifers from d 2 through 14 (Fig. 2) and concentrations of IGF-I were greater ($P < 0.001$) for 1.2M compared with 0.4M heifers during d 2 through 14 (Fig. 3). Concentrations of glucose and insulin were not influenced ($P > 0.10$) by treatment (Fig. 2); however, 0.4M heifers had less ($P < 0.01$) thyroxine in plasma during the entire sampling period compared with 1.2M heifers (Fig. 2). Concentrations of leptin in plasma during the total sampling period tended ($P = 0.10$) to be 23% less for 0.4M heifers compared with 1.2 M heifers (Fig. 3).

Five of the seven 0.4M heifers that failed to ovulate during restricted feeding resumed normal luteal cycles

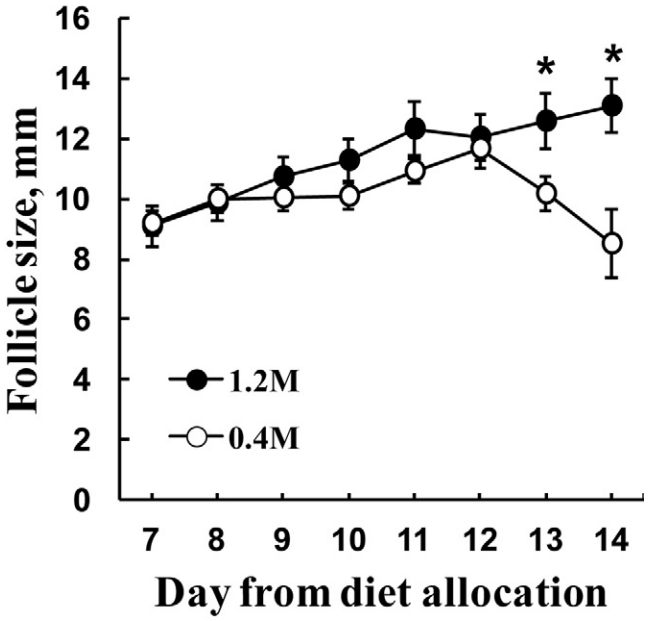

Figure 1. Size of the largest follicle on the ovary of heifers in Exp. 1 fed 1.2 or 0.4 times their maintenance energy requirement (1.2M and 0.4M, respectively; treatment × day, $P < 0.09$; *$P < 0.05$).

by 35 ± 6 d of realimentation (range = 16 to 51 d). Two of the seven 0.4M heifers that became anovulatory during restricted feeding failed to resume normal luteal activity by 100 d of realimentation. Concentrations of IGF-I in plasma of 0.4M heifers increased ($P < 0.05$) during realimentation and within 14 d did not differ ($P = 0.20$) compared with heifers maintained at 1.2M for the entire experiment (Fig. 4). During realimentation, concentrations of leptin in plasma did not differ ($P > 0.1$) between 0.4M and 1.2M heifers (Fig. 4).

Experiment 2

Body weight on d 0 did not differ ($P = 0.93$) for 0.4M and 1.2M heifers; however, 1.2M heifers had greater ($P < 0.01$) BW than 0.4M heifers on d 12 (Table 2). Heifers fed 0.4M lost 35 ± 4 kg during treatment, but BCS was similar for heifers on both treatments on d 0 ($P = 0.57$) and d 12 ($P = 0.19$; Table 2). On the basis of concentrations of progesterone in plasma from d 14 to

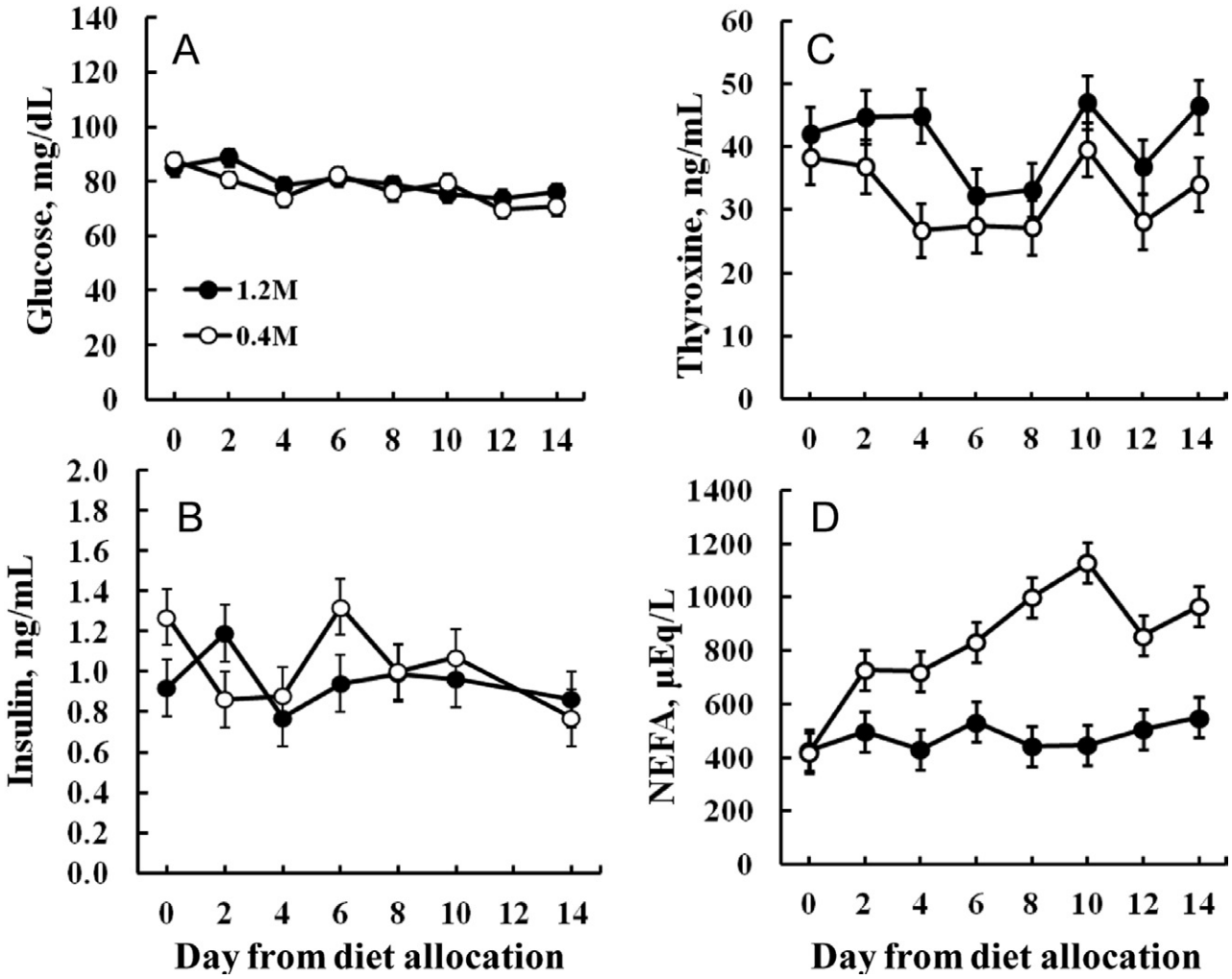

Figure 2. Least squares means for concentrations of (A) glucose, (B) insulin, (C) thyroxine (treatment, $P < 0.01$), and (D) NEFA (treatment × day, $P < 0.01$) in plasma of heifers in Exp. 1 fed 1.2 or 0.4 times maintenance energy requirement (1.2M and 0.4M, respectively).

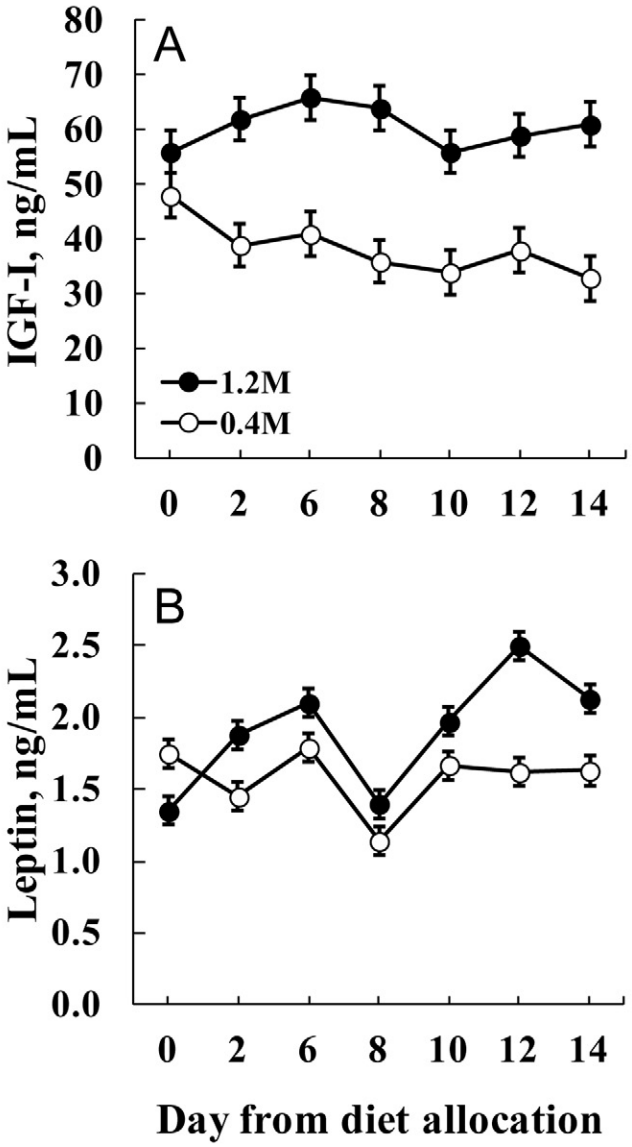

Figure 3. Least squares means for concentrations of (A) IGF-I (treatment × day, $P < 0.0001$) and (B) leptin (treatment × day, $P > 0.1$) in plasma of heifers in Exp. 1 fed 1.2 or 0.4 times their maintenance energy requirement (1.2M and 0.4M, respectively).

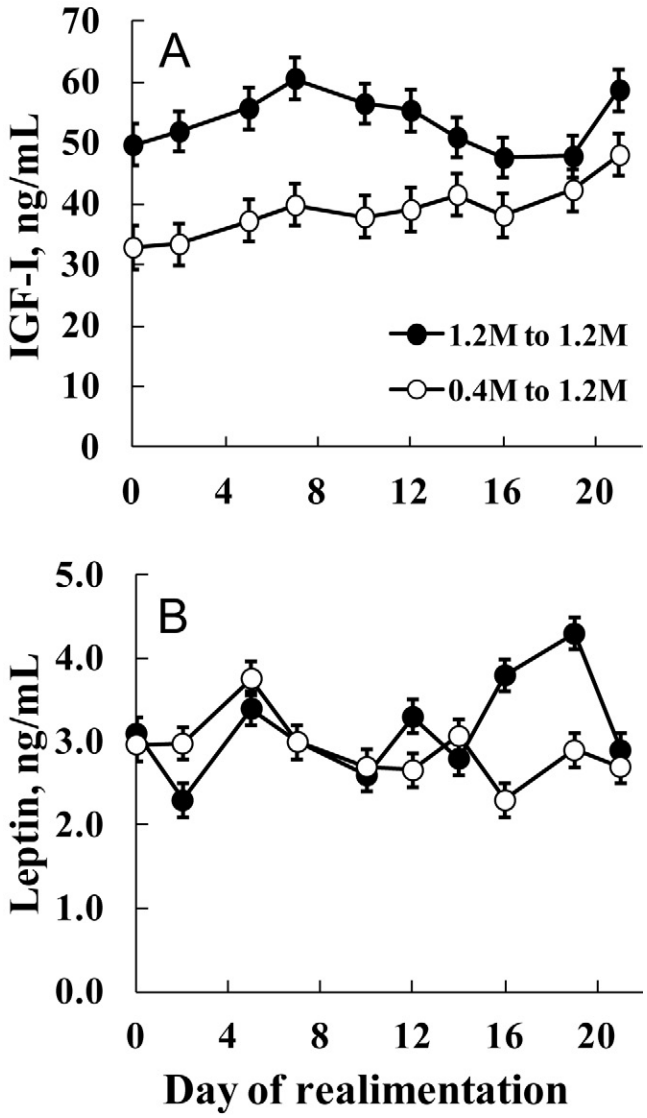

Figure 4. Least squares means for concentrations of (A) IGF-I (treatment × day, $P < 0.05$) and (B) leptin (treatment × day, $P > 0.1$) in plasma of heifers in Exp. 1 during realimentation. Heifers were fed either 1.2 or 0.4 times their maintenance energy requirement (1.2M and 0.4M, respectively) for 29 d. Beginning on d 0 of realimentation, 1.2M heifers were fed 1.2M for 90 d, and restricted heifers were fed 0.6M for 5 d, 0.8M for 5 d, then 1.2 M for 80 d.

21, all of the 1.2M heifers ovulated (4 of 4), whereas 56% (5 of 9) 0.4M heifers ovulated ($P = 0.10$; Table 2).

There was a treatment × day effect for plasma concentrations of NEFA ($P < 0.001$) and insulin ($P < 0.05$) but not for glucose ($P = 0.53$) or IGF-I ($P = 0.17$). The 0.4M heifers had greater ($P < 0.001$) concentrations of NEFA in plasma than 1.2M heifers from d 2 to 14 (Fig. 5). Concentrations of insulin for 0.4M heifers were less ($P < 0.05$) compared with 1.2M heifers (Fig. 5). There was a tendency ($P = 0.10$) for mean concentrations of glucose to be greater during the entire sampling period for 1.2M heifers compared with 0.4M heifers (76.1 ± 1.2 vs. 73.8 ± 0. 8 mg/dL, respectively). Concentrations of IGF-I were not different for 1.2M compared with 0.4M heifers (Fig. 5).

Mean concentrations of LH in serum on d 9, 10, and 11 (Table 3) were not influenced by treatment ($P = 0.88$) or treatment × day ($P = 0.80$). Mean concentrations of LH were greater ($P < 0.01$) for all heifers on d 10 and 11 (6.2 ± 0.4 and 6.4 ± 0.5 ng/mL, respectively) than on d 9 (4.7 ± 0.4 ng/mL. There was a treatment × day effect ($P < 0.05$) on frequency of LH pulses. Frequency of LH pulses was greater ($P < 0.05$) for 1.2M heifers compared with 0.4M heifers on d 9, but the number of LH pulses was not influenced ($P = 0.58$) by treatment on d 10 and 11 (Table 3). There was not a treatment × day effect ($P = 0.50$) on amplitude of LH pulses, but LH pulse amplitude for 0.4M and 1.2M heifers was greater ($P < 0.01$) on d 10 and 11 than on d 9.

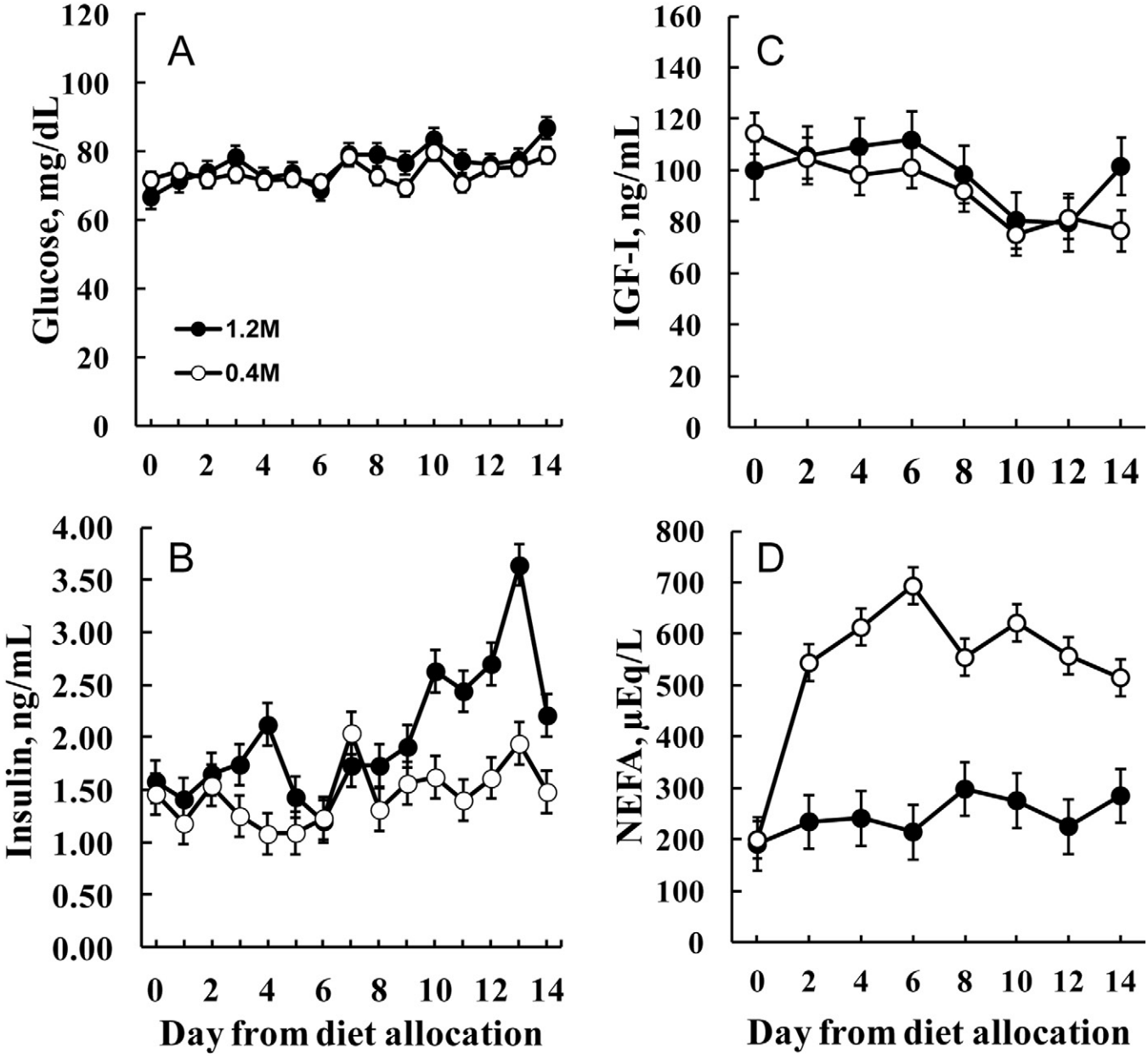

Figure 5. Least squares means for concentrations of (A) glucose (day, $P < 0.001$), (B) insulin (treatment × day, $P < 0.001$), (C) IGF-I (day, $P < 0.01$), and (D) NEFA (treatment × day, $P < 0.001$) in plasma of heifers from Exp. 2 that were fed 1.2 or 0.4 times their maintenance energy requirement (1.2M and 0.4M, respectively).

Table 3. Mean concentration, pulse frequency, and amplitude of luteinizing hormone in serum of heifers in Exp. 2 on d 9, 10, and 11 from diet allocation

	Dietary treatment[1]						SEM
	1.2M			0.4M			
Variable	d 9	d 10[2]	d 11	d 9	d 10[2]	d 11	
Mean concentration, ng/mL	4.8[a]	6.0[b]	6.5[b]	4.5[a]	6.3[b]	6.3[b]	0.6
Pulse frequency, pulses/8 h	5.0[a]	4.8[a]	4.7[a]	3.7[b]	4.5[a]	4.6[a]	0.2
Pulse amplitude, ng/mL	2.3[a]	3.6[b]	3.6[b]	2.3[a]	3.2[b]	3.3[b]	0.5

[a,b]Means within a row without a common superscript are different ($P < 0.05$).

[1]Animals received 1.2 times (1.2M) or 0.4 times (0.4M) their maintenance energy requirement for 21 d.

[2]Luteolysis was induced on d 10 with $PGF_{2\alpha}$.

Treatment did not influence ($P = 0.65$) concentrations of FSH in serum or amplitude ($P = 0.96$) or frequency ($P = 0.65$) of FSH pulses on d 11 (Table 4). Treatment did not influence concentrations of estradiol in plasma on d 11 ($P = 0.65$; Table 4).

An increase in concentrations of estradiol in plasma at proestrus was detected in 50% (2 of 4) of 1.2M heifers and 80% (4 of 5) 0.4M heifers that ovulated. An increase in estradiol at proestrus was not detected for heifers (0 of 4) fed 0.4M that failed to ovulate (Fig. 6; $P = 0.05$). Mean concentrations of estradiol during 48 to 72 h after $PGF_{2\alpha}$ were not different ($P = 0.40$) for 0.4M heifers that were anovulatory (1.1 ± 0.4 pg/mL) compared with 1.2M and 0.4M heifers that ovulated (1.8 ± 0.4 pg/mL). A

Table 4. Mean concentration, pulse frequency, and amplitude of follicle-stimulating hormone pulses in serum and estradiol in plasma on heifers in Exp. 2 on d 11 from diet allocation

	Dietary treatment[1]		
Variable	1.2M	0.4M	SEM
Mean concentration, ng/mL	0.22	0.27	0.07
Pulse frequency, pulses/8 h	5.3	5.8	0.6
Pulse amplitude, ng/mL	0.10	0.10	0.02
Estradiol, pg/mL	1.9	1.6	0.4

[1]Animals received 1.2 times (1.2M) or 0.4 times (0.4M) their maintenance energy requirement for 21 d.

preovulatory surge of LH was detected in 25% (1 of 4) of 1.2M heifers and 80% (4 of 5) of 0.4M heifers that ovulated. An ovulatory surge of LH was not detected ($P = 0.07$) in any of the 0.4M heifers that did not ovulate (Fig. 6).

DISCUSSION

Acute nutritional restriction resulted in ovulatory failure, based on concentration of progesterone in plasma, in 44% to 70% of heifers. Nutritional restriction altered the availability of oxidizable metabolic fuels (NEFA) in Exp. 1 and 2 and a tendency for reduced glucose in plasma in Exp. 2. Concentrations of IGF-I in plasma of 0.4M heifers were decreased compared with 1.2M heifers. Restriction of nutrients decreased LH pulse frequency before initiation of luteolysis, but growth rate and size of the dominant follicle were not different between treatments until after luteolysis had commenced.

Seventy percent of 0.4M heifers in Exp. 1 and 44% of 0.4M heifers in Exp. 2 failed to ovulate. This is similar to the 60% reported by Mackey et al. (1999). More recently, Walsh et al. (2012a) found that 35% of 0.4M heifers failed to ovulate. Lents et al. (2011) reported that 7% of 0.4M heifers became anovulatory between 14 and 21 d of treatment. Although the design of the experiment (Lents et al., 2011) did not have nonrestricted control heifers, 22% of the restricted heifers had abnormal luteal function based on plasma concentrations of progesterone in frequent blood samples during treatment, indicating that restriction of nutrient intake influenced ovarian function. Heifers in the current studies were fed a diet supplying 0.4 times their maintenance energy requirement once daily. Lents et al. (2011) divided the 0.4M allowance into

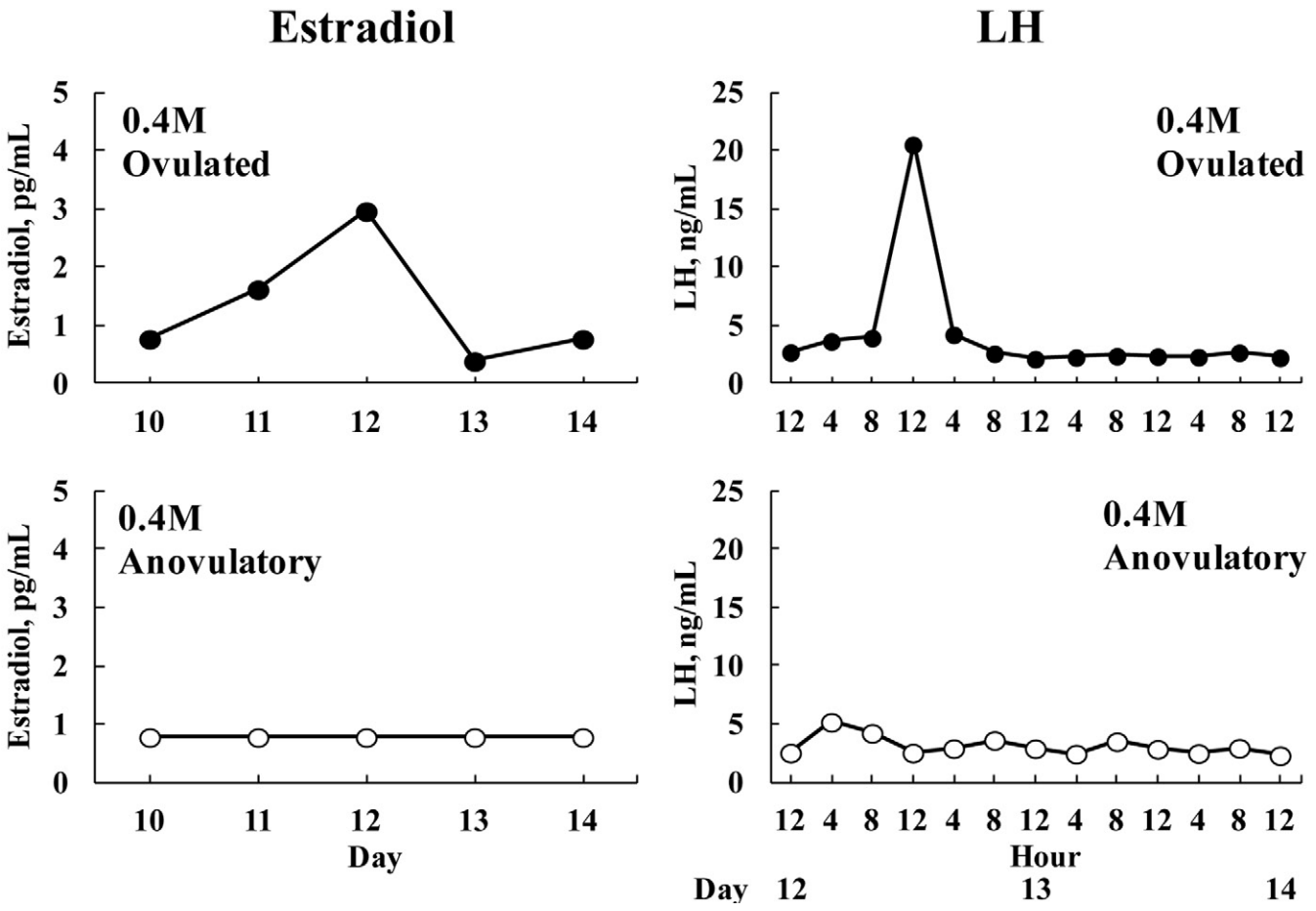

Figure 6. Individual profiles of estradiol in plasma and LH in serum on d 10 to 14 of treatment in 2 representative heifers fed 0.4 times their maintenance energy requirement (0.4M). One heifer ovulated and the other became anovulatory (based on plasma concentrations of progesterone through d 21). Luteolysis was induced on d 10 with $PGF_{2\alpha}$.

2 equal meals fed at the beginning and end of each day. Twice-daily feeding used by Lents et al. (2011) may have minimized the detrimental effects of the 0.4M diet on ovarian function, allowing more heifers to ovulate during restriction. Nonetheless, the level of nutrient restriction imposed here and in the aforementioned experiments caused significant loss of BW.

In agreement with the current studies, Mackey et al. (1999, 2000) observed that BCS was not altered by feeding 0.4M. Heifers lost approximately 2 mm of fat over the 13th rib after feeding the 0.4M diet for 21 d (Lents et al., 2011). Body condition score is a useful tool to determine major differences in body fatness of cattle (Wagner et al., 1988) but probably is not adequately sensitive to detect smaller changes in fat thickness over the short time period in these studies. Greater concentrations of NEFA in plasma of 0.4M heifers compared with 1.2M heifers agree with Mackey et al. (2000) and confirm that animals were mobilizing body fat to meet cellular demands for energy.

Metabolic shifts in response to nutrient restriction occur more rapidly in young growing females than in fully mature females (Foster et al., 1989; Barb et al., 1997; Amstalden et al., 2000). Metabolic hormones impact the hypothalamic-pituitary-ovarian axis and may determine the ovulatory response to 0.4M feeding (Mackey et al., 2000). In Exp. 1, we sought to establish the metabolic profile of heifers restricted to 0.4M or fed 1.2M. Concentrations of thyroxine in plasma reflect overall metabolic state and feed intake and are positively correlated with concentrations of IGF-I, leptin, insulin, and glucose in cattle (Ciccioli et al., 2003). The effect of acute nutritional restriction on concentrations of thyroxine in plasma of heifers during follicular growth has not been evaluated. Concentrations of thyroxine were less in 0.4M heifers, but concentrations of glucose and insulin were not influenced by treatment in Exp. 1. In Exp. 2, plasma concentrations of insulin were greater in 1.2M heifers; the treatment × day effect was associated with greater insulin in the 1.2M heifers during d 10 to 14 of treatment and not a decrease in insulin in the 0.4M heifers. The cause of the increase in insulin in the 1.2M heifers is not apparent. Variation in the response may be associated with the fact that blood samples were collected immediately before feeding in the morning. Insulin in plasma was not different between 1.2M and 0.4M heifers when blood samples were collected before feeding, but 1.2M heifers had greater concentrations of insulin in plasma than 0.4M heifers when blood samples were collected after feeding (Mackey et al., 2000). Furthermore, Walsh et al. (2012a,b) found decreased insulin in plasma of 0.4M heifers compared with heifers fed 1.2M.

Reduced growth of dominant follicles and decreased plasma concentrations of IGF-I were associated with nutritionally induced cessation of ovulation of beef heifers (Bossis et al., 1999). In Exp. 1, concentrations of IGF-I in plasma declined in 0.4M heifers but were maintained in 1.2M heifers, which is consistent with previous reports (Mackey et al., 1999, 2000; Lents et al., 2011). Plasma concentrations of IGF-I increased in 0.4M heifers during realimentation; however, IGF-I was not influenced by treatment in Exp. 2. Previous nutrient intake, or days after the onset of pubertal estrous cycles, could influence the effect of nutrient restriction on plasma concentrations of IGF-I. Animals in Exp. 1 and 2 were genetically similar; however, BW of heifers was greater (66 kg) in Exp. 2 compared with Exp. 1 and may be associated with the absence of a treatment effect on plasma concentrations of IGF-I. Insulin-like growth factor-I has an important role in mitogenic growth and steroidogenic capacity of granulosa cells (Spicer and Echternkamp, 1995; Spicer, 2004; Velazquez et al., 2008). Follicular concentrations of IGF-I and expression of IGFBP-2 mRNA in thecal cells of dominant follicles from 0.4M heifers were less than in dominant follicles of 1.2M heifers (Walsh et al., 2012a). Intrafollicular concentrations of IGF-I were greater in dominant follicles of 0.4M heifers that were anovulatory, and granulosa cells from dominant follicles of anovulatory 0.4M heifers had reduced expression of mRNA for IGF-2 and IGF-I receptor compared with dominant follicles from ovulatory 0.4M heifers (Walsh et al., 2012a). Differences in protein concentrations of these components of the IGF system were not determined (Walsh et al., 2012a). Serum from nutritionally anovulatory heifers stimulates less proliferation of bovine granulosa cells in vitro than serum from cyclic control heifers (Spicer et al., 2008). Mitogenic activity of the serum from nutritionally anovulatory heifers was restored when IGF-I was added to culture but not to the same extent as when IGF-I was added to granulosa cells cultured with serum from the cyclic control heifers (Spicer et al., 2008). This is likely because nutritional restriction increases serum concentrations of IGFBP-2 in cattle (Vandehaar et al., 1995). Systemically produced IGF-I and IGFBP, along with ovarian synthesis of these factors, is important for regulating granulosa cell function during follicular growth. Therefore, the reduced size of the dominant follicles in 0.4M heifers in Exp. 1 may have been a result of reduced proliferation of granulosa cells because plasma concentrations of IGF-I and probably intrafollicular concentrations of IGF-I and IGFBP were altered.

Concentrations of leptin in plasma of gestating beef cows indicate the adequacy of nutrient intake but are not associated with body energy stores (Lents et al., 2005). The absence of a major effect of acute nutritional restriction on concentrations of leptin in plasma of heifers in the current experiment indicates that it is not a metabolic signal that regulates secretion of gonadotropins.

Mackey et al. (1999) speculated that failure of ovulation in heifers fed 0.4M resulted from altered pulsatile secretion of LH during either emergence, selection, or dominance phases of the follicular wave. These times correspond to approximately d 4, 8, and 12 in the current experiment. Pulses of LH occurred less frequently in 0.4M heifers in the current experiment before induction of luteal regression. Diet-induced changes in LH pulse frequency before luteolysis might have impacted the ovulatory response to nutritional treatment; however, after luteolysis was initiated with $PGF_{2\alpha}$, LH pulse frequency was similar for 0.4M and 1.2M heifers. Ovariectomized heifers fed 0.4M had greater mean concentrations of LH because of greater LH pulse amplitude compared with 1.2M heifers, but there was no effect of dietary restriction (0.4M) on pulsatile release of LH in ovary-intact heifers (Mackey et al., 2000). Walsh et al. (2012a) found decreased expression of LH receptor mRNA in the theca cells of 0.4M heifers that failed to ovulate. Even though there was sufficient LH secretion to support follicle growth, 0.4M heifers that failed to ovulate in the current study may have had follicles with reduced LH responsiveness due to the decrease in systemic IGF-I, as IGF-I increases thecal cell LH receptors in cattle (Stewart et al., 1995).

Ovulatory failure in heifers that were subjected to moderate (0.7M) but chronic nutritional restriction was accompanied by reduced concentrations of estradiol and increased concentrations of FSH in serum 48 h after $PGF_{2\alpha}$ treatment (Bossis et al., 1999). Basal concentrations of FSH in 0.4M heifers were not different from those for 1.2M heifers. In contrast, Mackey et al. (2000) found that concentrations of FSH tended to be greater in 0.4M heifers compared with 1.2M heifers. In the current experiment, FSH concentrations in serum and frequency and amplitude of FSH pulses 24 h after $PGF_{2\alpha}$ were not influenced by treatment. This reflects the fact that concentrations of estradiol in plasma at this time were similar for 0.4M and 1.2M heifers and indicates that dominant follicles of 0.4M heifers were effective at suppressing secretion of FSH.

The preovulatory increase in estradiol and the ovulatory surge of LH were detected in some of the 1.2M heifers and in most of the 0.4M heifers that ovulated. Because heifers were confined to stalls, we were unable to determine when estrus began after induction of luteolysis, so we quantified estradiol and LH every 4 h beginning at 1200 h on d 12 until 1200 h on d 14. An LH surge was detected in 80% of the 0.4M heifers that ovulated. All of the 1.2M heifers ovulated, but a proestrus increase in estradiol was detected in only 50% of the heifers, and an ovulatory surge of LH was detected in 25% of the heifers. On the basis of concentrations of progesterone in plasma, the 1.2M heifers did not ovulate until probably after d 14, or 96 h after treatment with $PGF_{2\alpha}$. A previous experiment (Bossis et al., 1999) found that plasma concentrations of progesterone and estradiol were not different, compared with control heifers, when $PGF_{2\alpha}$ was given to cause luteal regression during the last 2 estrous cycles before nutritionally induced anovulation. Thus, there was no reason to anticipate that the preovulatory increase in estradiol would occur greater than 72 h after treatment with $PGF_{2\alpha}$. However, our sampling protocol was not sufficient to detect preovulatory changes in estradiol or LH in these heifers.

Proestrus increases in estradiol, or a preovulatory surge of LH, were not detected in any of the 0.4M heifers that did not ovulate. This supports the assertion that dominant follicles in 0.4M heifers, in which ovulatory failure occurs, are not capable of secreting adequate estrogen to stimulate an ovulatory surge of LH (Mackey et al., 1999). Walsh et al. (2012b) found less IGF-I and estradiol in follicular fluid collected from mature dominant follicles from 0.4M heifers than from 1.2M heifers. This was associated with reduced mRNA expression for steroidogenic acute regulatory protein (**STAR**), a mitrochondrial phosphoprotein that is critical in the transport of cholesterol for steroidogenesis (Stocco, 2000; Niswender, 2002), in thecal cells of dominate follicles of 0.4M heifers; abundance of STAR mRNA was correlated with concentrations of IGF-I in serum (Walsh et al., 2012b). In addition, a reduction in systemic IGF-I could also reduce aromatization of thecal androgens into estrogens by granulosa cells (Spicer et al., 2002). Therefore, reduced concentrations of IGF-I in plasma of heifers in the current study may have compromised the ability of the follicle to produce sufficient steroids to generate a preovulatory surge of estradiol and initiate ovulation.

Realimentation of the 0.4M heifers to the 1.2M diet resulted in resumption of ovulation in 71% of heifers within approximately 35 d. The interval from realimentation to first ovulation was 57 to 80 d in heifers after exposure to modest (0.7M) but chronic nutritional restriction (Bossis et al., 2000). Although the degree of nutrient restriction in the current study was greater, the duration until ovulation was not as long because 0.4M heifers did not deplete body energy reserves to the same extent as those in Bossis et al. (2000). Greater concentrations of IGF-I in plasma are associated with a shorter interval from parturition to onset of estrous cycles in beef cows (Rutter et al., 1989; Roberts et al., 1997; Lents et al., 2008) and in nutritionally induced anovulatory heifers during realimentation (Bossis et al., 2000). Plasma concentrations of IGF-I were increased a few days after commencing realimentation in this study, and a shorter interval was required for resumption of ovarian luteal activity compared with other studies in which greater intervals occurred before heifers became anovulatory (Bossis et al., 2000).

Duration to the reinitiation of normal ovarian function when nutritionally induced anovulatory animals received

adequate nutrient intake may be influenced by differences between animals as to the requirements for maintenance and growth. Vizcarra et al. (1995) individually fed heifers NRC requirements (NRC, 1976) from 8 mo of age until puberty at an average age of 423 d, then diets were restricted; heifers that were the first to attain puberty were the last to cease luteal activity during nutritional restriction. Variation occurs in the interval from realimentation of nutritionally induced anovulatory until reinitiation of ovarian function in heifers (Imakawa et al., 1986; Bossis et al., 2000) and cows (Richards et al., 1989).

In summary, restricting young beef heifers to 0.4 × maintenance energy requirements acutely altered the availability of metabolic fuels and reduced circulating concentrations of IGF-I. The frequency of LH pulses before luteolysis was reduced and the size of the dominant follicle was suppressed in 0.4M heifers. These events resulted in the absence of a preovulatory increase in estradiol and the ovulatory surge of LH, resulting in ovulatory failure in 44% to 70% of heifers. The biological basis for some heifers to remain cyclic during nutrient restriction requires further investigation.

LITERATURE CITED

Amstalden, M., M. R. Garcia, S. W. Williams, R. L. Stanko, S. E. Nizielski, C. D. Morrison, D. H. Keisler, and G. L. Williams. 2000. Leptin gene expression, circulating leptin, and luteinizing hormone pulsatility are acutely responsive to short-term fasting in prepubertal heifers: Relationships to circulating insulin and insulin-like growth factor I. Biol. Reprod. 63:127–133.

Barb, C. R., R. R. Kraeling, G. B. Rampacek, and C. R. Dove. 1997. Metabolic changes during the transition from the fed to the acute feed-deprived state in prepuberal and mature gilts. J. Anim. Sci. 75:781–789.

Bishop, D. K., and R. P. Wettemann. 1993. Pulsatile infusion of gonadotropin-releasing hormone initiates luteal activity in nutritionally anestrous beef cows. J. Anim. Sci. 71:2714–2720.

Bossis, I., R. P. Wettemann, S. D. Welty, J. Vizcarra, and L. J. Spicer. 2000. Nutritionally induced anovulation in beef heifers: Ovarian and endocrine function during realimentation and resumption of ovulation. Biol. Reprod. 62:1436–1444.

Bossis, I., R. P. Wettemann, S. D. Welty, J. A. Vizcarra, L. J. Spicer, and M. G. Diskin. 1999. Nutritionally induced anovulation in beef heifers: Ovarian and endocrine function preceding cessation of ovulation. J. Anim. Sci. 77:1536–1546.

Ciccioli, N. H., R. P. Wettemann, L. J. Spicer, C. A. Lents, F. J. White, and D. H. Keisler. 2003. Influence of body condition at calving and postpartum nutrition on endocrine function and reproductive performance of primiparous beef cows. J. Anim. Sci. 81:3107–3120.

Day, M. L., K. Imakawa, D. D. Zalesky, R. J. Kittok, and J. E. Kinder. 1986. Effects of restriction of dietary energy intake during the prepubertal period on secretion of luteinizing hormone and responsiveness of the pituitary to luteinizing hormone-releasing hormone in heifers. J. Anim. Sci. 62:1641–1648.

Delavaud, C., F. Bocquier, Y. Chilliard, D. H. Keisler, A. Gertler, and G. Kann. 2000. Plasma leptin determination in ruminants: Effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. J. Endocrinol. 165:519–526.

Diskin, M. G., D. R. Mackey, J. F. Roche, and J. M. Sreenan. 2003. Effects of nutrition and metabolic status on circulating hormones and ovarian follicle development in cattle. Anim. Reprod. Sci. 78:345–370.

Echternkamp, S. E., L. J. Spicer, K. E. Gregory, S. F. Canning, and J. M. Hammond. 1990. Concentrations of insulin-like growth factor-I in blood and ovarian follicular fluid of cattle selected for twins. Biol. Reprod. 43:8–14.

Federation of Animal Science Societies. 2010. Guide for the care and use of agricultural animals in research and teaching. 3rd ed. Fed. Anim. Sci. Soc., Champaign, IL.

Foster, D. L., F. J. P. Ebling, A. F. Micka, L. A. Vannerson, D. C. Bucholtz, R. I. Wood, J. M. Suttie, and D. E. Fenner. 1989. Metabolic interfaces between growth and reproduction. I. Nutritional modulation of gonadotropin, prolactin, and growth hormone secretion in the growth-limited female lamb. Endocrinology 125:342–350.

Garrett, W. N. 1980. Energy utilization by growing cattle as determined in 72 comparative slaughter experiments. Energy Metab. Proc. Symp. 26:3–7.

Imakawa, K., M. L. Day, M. Garcia-Winder, D. D. Zalesky, R. J. Kittok, B. D. Schanbacher, and J. E. Kinder. 1986. Endocrine changes during restoration of estrous cycles following induction of anestrus by restricted nutrient intake in beef heifers. J. Anim. Sci. 63:565–571.

Lents, C. A., R. D. Randel, A. M. Stelzleni, L. C. Caldwell, and T. H. Welsh Jr. 2011. Function of the corpus luteum in beef heifers is affected by acute submaintenance feeding but is not correlated with residual feed intake. J. Anim. Sci. 89:4023–4031.

Lents, C. A., R. P. Wettemann, F. J. White, I. Rubio, N. H. Ciccioli, L. J. Spicer, D. H. Keisler, and M. E. Payton. 2005. Influence of nutrient intake and of body fat on concentrations of insulin-like growth factor-I, insulin, thyroxine, and leptin in plasma of gestating beef cows. J. Anim. Sci. 83:586–596.

Lents, C. A., F. J. White, N. H. Ciccioli, R. P. Wettemann, L. J. Spicer, and D. L. Lalman. 2008. Effects of body condition score at parturition and postpartum protein supplementation on estrous behavior and size of the dominant follicle in beef cows. J. Anim. Sci. 86:2549–2556.

Mackey, D. R., J. M. Sreenan, J. F. Roche, and M. G. Diskin. 1999. Effect of acute nutritional restriction on incidence of anovulation and periovulatory estradiol and gonadotropin concentrations in beef heifers. Biol. Reprod. 61:1601–1607.

Mackey, D. R., A .R. G. Wylie, J. M. Sreenan, J. F. Roche, and M. G. Diskin. 2000. The effect of acute nutritional change on follicle wave turnover, gonadotropin, and steroid concentration in beef heifers. J. Anim. Sci. 78:429–442.

McCutcheon, S. N., and D. E. Bauman. 1986. Effect of chronic growth hormone treatment on responses to epinephrine and thyrotropin-releasing hormone in lactating cows. J. Dairy Sci. 69:44–51.

Merriam, G. R., and K. W. Wachter. 1982. Algorithms for the study of episodic hormone secretion. Am. J. Physiol. 243:E310–E318.

Niswender, G. D. 2002. Molecular control of luteal secretion of progesterone. Reproduction 123:333–339.

NRC. 1976. Nutrient requirements of beef cattle. 5th ed. Natl. Acad. Press, Washington, DC.

Rhodes, F. M., K. W. Entwistle, and J. E. Kinder. 1996. Changes in ovarian function and gonadotropin secretion preceding the onset of nutritionally induced anestrus in *Bos indicus* heifers. Biol. Reprod. 55:1437–1443.

Richards, M. W., R. P. Wettemann, and H. M. Schoenemann. 1989. Nutritional anestrus in beef cows: Body weight change, body condition, luteinizing hormone in serum and ovarian activity. J. Anim. Sci. 67:1520–1526.

Roberts, A. J., R. A. Nugent III, J. Klindt, and T. G. Jenkins. 1997. Circulating insulin-like growth factor I, insulin-like growth factor binding proteins, growth hormone, and resumption of estrus in postpartum cows subjected to dietary energy restriction. J. Anim. Sci. 75:1909–1917.

Rutter, L. M., R. Snopek, and J. G. Manns. 1989. Serum concentrations of IGF-I in postpartum beef cows. J. Anim. Sci. 67:2060–2066.

Spicer, L. J. 2004. Proteolytic degradation of insulin-like growth factor binding proteins by ovarian follicles: A control mechanism for selection of dominant follicles. Biol. Reprod. 70:1223–1230.

Spicer, L. J., I. Bossis, and R. P. Wettemann. 2008. Effect of plasma from cyclic versus nutritionally induced anovulatory beef heifers on proliferation of granulosa cells in vitro. Domest. Anim. Endocrinol. 34:250–253.

Spicer, L. J., C. S. Chamberlain, and S. M. Maciel. 2002. Influence of gonadotropins on insulin- and insulin-like growth factor-I (IGF-I)-induced steroid production by bovine granulosa cells. Domest. Anim. Endocrinol. 22:237–254.

Spicer, L. J., and S. E. Echternkamp. 1995. The ovarian insulin and insulin-like growth factor system with an emphasis on domestic animals. Domest. Anim. Endocrinol. 12:223–245.

Stewart, R. E., L. J. Spicer, T. D. Hamilton, and B. E. Keefer. 1995. Effects of insulin-like growth factor I and insulin on proliferation and on basal and luteinizing hormone-induced steroidogenesis of bovine thecal cells: Involvement of glucose and receptors for insulin-like growth factor I and luteinizing hormone. J. Anim. Sci. 73:3719–3731.

Stocco, D. M. 2000. The role of the StAR protein in steroidogenesis: Challenges for the future. J. Endocrinol. 164:247–253.

Vandehaar, M. J., B. K. Sharma, and R. L. Fogwell. 1995. Effect of dietary energy restriction on the expression of insulin-like growth factor-I in liver and corpus luteum of heifers. J. Dairy Sci. 78:832–841.

Velazquez, M. A., L. J. Spicer, and D. C. Wathes. 2008. The role of endocrine insulin-like growth factor-I (IGF-I) in female bovine reproduction. Domest. Anim. Endocrinol. 35:325–342.

Vizcarra, J. A., R. P. Wettemann, and D. K. Bishop. 1995. Relationship between puberty in heifers and the cessation of luteal activity after nutritional restriction. Anim. Sci. 61:507–510.

Vizcarra, J. A., R. P. Wettemann, T. D. Braden, A. M. Turzillo, and T. M. Nett. 1997. Effect of gonadotropin-releasing hormone (GnRH) pulse frequency on serum and pituitary concentrations of luteinizing hormone and follicle-stimulating hormone, GnRH receptors, and messenger ribonucleic acid for gonadotropin subunits in cows. Endocrinology 138:594–601.

Wagner, J. J., K. S. Lusby, J. W. Oltjen, J. Rakestraw, R. P. Wettemann, and L. E. Walters. 1988. Carcass composition in mature Hereford cows: Estimation and effect on daily metabolizable energy requirement during winter. J. Anim. Sci. 66:603–612.

Walsh, S. W., D. Matthews, J. A. Browne, N. Forde, M. A. Crowe, M. Mihm, M. Diskin, and A. C. O. Evans. 2012a. Acute dietary restriction in heifers alters expression of genes regulating exposure and response to gonadotrophins and IGF in dominant follicles. Anim. Reprod. Sci. 133:43–51.

Walsh, S. W., J. P. Mehta, P. A. McGettigan, J. A. Browne, N. Forde, R. M. Alibrahim, F. J. Mulligan, B. Loftus, M. A. Crowe, D. Matthews, M. Diskin, M. Mihm, and A. C. O. Evans. 2012b. Effect of the metabolic environment at key stages of follicle development in cattle: Focus on steroid biosynthesis. Physiol. Genomics 44:504–517.

Wettemann, R. P., and I. Bossis. 2000. Energy intake regulates ovarian function in beef cattle. J. Anim. Sci. 77(E-Suppl.):1–10.

Wettemann, R. P., H. D. Hafs, L. A. Edgerton, and L. V. Swanson. 1972. Estradiol and progesterone in blood serum during the bovine estrous cycle. J. Anim. Sci. 34:1020–1024.

White, F. J., I. Rubio, C. A. Lents, N. H. Ciccioli, R. P. Wettemann, and L. J. Spicer. 2008. Effect of days after calving on insulin-like growth factor-I, insulin-like growth factor binding proteins, progesterone, androstenedione, estradiol, and aromatase mRNA in dominant follicles of postpartum beef cows. Anim. Reprod. Sci. 108:364–374.